EUDRAGIT COATED SODIUM ALGINATE MICROSPHERES OF ACECLOFENAC FOR PAIN MANAGEMENT IN OSTEOARTHRITIS

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ABSTRACT

In the present study, microspheres of aceclofenac using sodium alginate were prepared and coated with eudragit to extend the release. The particle size of uncoated sodium alginate microsphere showed narrow variability ranging from 88.5 to 88.9 µm. It was observed that mean particle size of microspheres increased after coating with Eudragit RS 100 polymer i.e., 685.5 ± 9.0 µm. The optimized eudragit coated alginate microspheres were found to possess good entrapment efficiency. In vitro drug release of optimized formulation during 8 hours of study was found to be 20%, 48% and 72% in pH progression medium of 1.2, 6.8 and 7.4 pH respectively. The release profile would follow zero-order kinetics. Optimized coated microsphere potentially offers extended release of drug particularly for pain management. A highly significant (p < 0.05) anti-inflammatory action of the treatment of optimized coated alginate microsphere of aceclofenac was evidenced by inhibition of rat paw edema.

Keywords: Aceclofenac, Sodium Alginate, Eudragit, Microsphere, Pain Management

INTRODUCTION

The microparticles system is a promising strategy to achieve modified release of many drugs [1], have gained great interest in oral drug delivery, as they show several advantages over single-unit dosage forms, such as lower variability in the GI transit times, better drug dispersion, and the possibility of mixing particles with different release properties [2-3].

In addition to sustained delivery of drug, and thus, prolonged effect of the drug with a single administration, these systems have
also been demonstrated to influence the bioavailability of the drug, less fluctuation in drug blood level, reduction frequency in dosing and adverse effects and enhanced convenience and compliance with treatment [4].

In recent years, biotechnology and pharmaceutical industries have shown increased interest in the use of biodegradable polymers for tissue engineering and controlled drug delivery. Various polymers have been introduced in drug delivery research as they can effectively deliver the drug to a target site and thus increase the therapeutic benefit and minimizing side effects [5-6].

Alginates are haemo-compatible and did not accumulate in any of the major organs [7]. The quality of the alginates has improved in the recent years due to advancement in technology. Alginates are used in different areas such as matrixing agent, encapsulating agent etc. The cross-linking of alginate is a function of both to the alginate composition and the length of the molecule. The affinity of the cross-linking cation for the alginate is also of great importance in the crosslinking reaction. The preparation of alginate particles described here is based on the cross linking properties of this polysaccharide with CaCl₂. The encapsulated drug is released from the alginate matrix at a controlled rate [8].

Aceclofenac is a potent non-steroidal anti-inflammatory drug (NSAID) and used for the relief of pain and inflammation in rheumatoid arthritis, osteoarthritis and ankylosing spondylitis. Aceclofenac produces potentially life-threatening effects which include severe GI bleeding, peptic ulceration, hematotoxicity, which are responsible for the discontinuation of aceclofenac therapy. Apart from GI toxicity of aceclofenac, it is poorly water soluble due to which its dissolution in GI fluid is very low, which in turn adversely affect the bioavailability [9, 10].

Usual therapeutic dose of aceclofenac is 100 mg twice daily and half life is 3-4 hrs, thus it is necessary to be administered frequently in order to maintain the desired concentration. If inflammation is worsening, secondarily increasing pain, it is possible that increases in dose would be effective in controlling the greater pain. Unfortunately, high doses increases the risk of many serious adverse effects. Therefore, aceclofenac is an ideal candidate for sustained release formulation, resulting in more reproducible drug absorption and reducing the risk of local irritations compared to single dosage forms.

MATERIALS AND METHODS
Aceclofenac (ACF) was provided by Ranbaxy Research laboratory, India. Sodium alginate and Eudragit RS-100 were
purchased from G.S chemical testing & Allied India and Jubilant organosys, Noida, India respectively. Calcium chloride, span 80, tween 80, n-hexane, dichlormethane were purchase from S.d Fine Chem Ltd, India. light-liquid paraffin, corn oil, ethanol (95%) were purchased from Central Drug House, India, Chopra chemicals Pvt. Ltd, India, and Merck India Ltd., India respectively. All other chemicals used in the study were of analytical grade.

**Preparation of Sodium Alginate Microspheres**

The emulsification method was utilized for preparation of microspheres followed by cross linking with calcium chloride [11-13]. Weighed amount of drug was dispersed in 5 % aqueous solution of sodium alginate (10 ml). The aqueous phase was emulsified in light liquid paraffin in the ratio of 1: 4 containing 0.2 % (w/v) span 80 using mechanical stirrer (Remi Motors, India) at 1200 rpm for 2hr. Calcium chloride solution (7.5 %) as cross linking agent was added slowly to emulsion and stirred for another 5 min. The microspheres were collected by filtration, washed with distilled water and finally n-hexane after that dried in oven at 37±0.5 °C. Various variables like polymer concentration, drug-polymer ratio, concentration of cross-linking agent and time required for cross-linking were considered in the optimization of the formulation. On the basis of yield and entrapment efficiency F2 formulation code is selected for coating (Table 1).

**Coating of Sodium Alginate Microspheres**

The coating of optimized (F2) alginate microspheres was carried out by using liquid phase coating technique [14]. The 4% Eudragit RS 100 polymer solution in dichloromethane was dispersed in 100 ml of liquid paraffin containing 1% w/w Span 80, at a stirring speed of 8000 rpm. After 2-5 min, 100, 300 and 500 mg of core alginate (F2C1-F2C3) microspheres dispersed in 10 ml of light liquid paraffin were added to the above emulsion and stirring continued at 800 rpm for 5 min at room temperature to allow evaporation of dichloromethane. The coated alginate microspheres were collected by filtration, washed with 30 ml of n-hexane and dried in oven at 37±0.5 °C (Table 2).

**Morphological and Particle Size Study**

The shape and surface characteristic of placebo and drug loaded formulations were determined by scanning electron microscopy (SEM) using gold sputter technique [15]. The particles were vacuum dried, coated with gold palladium and observed microscopically. Particle sizes were measured by laser diffraction particle size analyzer (Mastersizer E, Malvern, UK).

**Differential Scanning Calorimetry**
Differential scanning calorimetry scans of pure drug and optimized coated formulation were recorded and compared. Sample containing 5 mg of drug and optimized formulation was placed in aluminium pan and heated from 50 °C to 300 °C at heating rate of 10°C/ min under inert atmosphere flushed with nitrogen at the rate of 20 ml /min [16].

**Determination of Drug Content and Entrapment Efficiency of Microspheres**

An appropriate amount of uncoated and coated alginate microspheres of aceclofenac were added to 25 ml of phosphate buffer with pH6.8 and absolute ethanol respectively. The samples were placed in an ultrasonic bath for three consecutive periods of 20 min, with 60 min of rest in between. The sample was then left to stand for overnight at room temperature. Then filtered the sample using 0.45µm filter and drug content was analyzed by assaying spectrophotometrically at 273 nm, 276 nm for aceclofenac respectively for both medium.

The drug content and entrapment efficiency of the microspheres were calculated as

\[ \% \text{ EE} = \left( \frac{\text{Actual Drug Content}}{\text{Theoretical Drug Content}} \times 100 \right) \]

**Drug Release Kinetics**

*In vitro* drug release from optimized coated formulation (F2C) was studied in the gradual pH change method [17]. The dissolution studies were carried out in 100 ml dissolution medium, which was stirred at 80 rpm at 37 ± 1°C. The change in the pH of solution was performed with respected to time with HCl buffer pH 1.2, phosphate buffer with pH 6.8 and pH 7.4 for 0-120 min, 180-300 minutes and 360-480 mins respectively. The release profile was fitted to various mathematical models representing Zero-order, First order, Higuchi’s and K. Peppas matrix.

**Anti-Inflammatory Studies: The Rat Paw Edema Method**

Approval to carry out *in vivo* studies was obtained from the Institutional Animal Ethics Committee, JamiaHamdard, New Delhi, India and their guidelines were followed for the studies. The anti-inflammatory and sustaining action of the optimized formulations were evaluated by carrageenan-induced hind paw edema method developed Wistar rats [18]. Young Wistar rats, weighing 150-250 g were randomly divided into 4 groups: control, ACF Pure powder, Marketed tablet and ACF microspheres formulation (F2C) each containing 6 rats. The animals were kept under standard laboratory conditions, at a temperature of 25 ± 1°C and relative humidity of 55 ± 5%. The animals were housed in polypropylene cages, six per cage, with free access to standard laboratory diet and water ad libitum. Dose for the rats was calculated based on the weight of the rats according to the surface area ratio [19].
Animals were starved 18 to 24 hr prior to administration of testing agents. Calculated dose of each formulation were administered orally to each group except control group which was administered 1 % CMC solution through p.o route, half an hour before subplanter injection of carrageenin in right paw. Paw edema was induced by injecting 0.1 ml of 1 % v/v homogeneous suspension of carrageenin in distilled water. The volume of paw was measured at 1, 2, 3, 6, 12 and 18 h after injection using digital plethysmometer. The amount of paw swelling was determined time to time and expressed as percent edema relative to the initial hind paw volume. Percent inhibition of edema produced by each formulation-treated group was calculated against the respective control group using the equation:

\[
\text{% Inhibition} = \frac{\% \text{Edema (control)} \times \% \text{Edema (formulation)}}{\% \text{Edema (control)}} \times 100
\]

**RESULT AND DISCUSSION**

**Morphological and Particle Size Study**

SEM was carried out in order to characterize surfacemorphology of the microspheres. In this study themorphological observations were carried out to study the surface morphology of microspheres. SEM photograph of Placebo, and coated alginate microspheres (F2C) are given in Figure 1. It was observed that Placebo were spherical in shape having porous surface while coated alginate microspheres (F2C) showed spherical in shape with smooth surface. No change in surface morphology was observed on drug loading.

Table 3 present the mean particle size, polydispersity index and entrapment efficiency for all the prepared formulae. The polydispersity index is a measure of the width of the dispersion of particles. A polydispersity index of 1 indicates large variations in particle size while a polydispersity value less than 0.1 is regarded as monodisperse. Narrow dispersions comprise polydispersity index values between 0.1 and 0.2.

The particle size of uncoated sodium alginate microsphere showed a narrow range of variability ranging from 88.5 to 88.9 µm. Eudragit coated optimized sodium alginate microsphere (F2C) have the particle size within the range of 685.5 ± 9.0 µm. The particle size distribution showed the maximum percentage of particles in the range of 686 to 688 µm while very low percentage of particles were less than 80-100 µm, the data showed that the maximum particle were coated, have uniform distribution.

**Differential Scanning Calorimetry**

The thermograms of pure drug and aceclofenac loaded microspheres are shown in Figure 2. The thermogram of aceclofenac showed a sharp endothermic peak at
153.438°C, which corresponds to its melting point. This peak was absent in the thermogram for the Aceclofenac loaded microspheres microsphere.

**Determination of Drug Content and Entrapment Efficiency of Microspheres**

On the basis of better yield and entrapment efficiency, the formulations F2 was selected as optimized core formulation but *in-vitro* released study of these formulations showed that alginate is not a suitable polymer for sustained release while it is easy to prepare with good yield. The release of optimized core formulation was modified by coating of release modifier such as eudragit (Table 3).

**Drug Release Kinetics**

The eudragit coated microspheres of alginate were subjected to *in vitro* drug release rate studies. Release rate were determined in pH progression medium of 1.2, 6.8 and 7.4 pH for 2 hours in each pH in order to investigate the capability of the formulation to withstand the physiological environment of the stomach and small intestine. *In vitro* drug release of optimized formulation during 8 hours of study was found to be 20%, 48% and 72% in pH progression medium of 1.2, 6.8 and 7.4 pH respectively (Figure 3).

Zero-order kinetics, First-order kinetics, Higuchi equation and Korsmeyer-Peppas models were used to determine the drug release kinetics. Kinetic assessments of the dissolution data are shown in Figure 4. In optimized formulation of eudragit coated sodium alginate aceclofenac microspheres in the release layer.

Since the coefficient of co-relation for Zero order release kinetic is higher than Higuchi’s, K.Peppas and first order release kinetic models, aceclofenac release from coated sodium alginate microspheres followed Zero order release kinetic. Zero order kinetics was found to be efficient in describing the kinetics of drug release, with fraction of drug release proportional to the time. Ideal delivery of drugs would follow “zero-order kinetics”, wherein blood levels of drugs would remain constant throughout the delivery period. This ideal delivery is particularly important in pain management [20].

**Anti-inflammatory Studies: The Rat Paw Edema Method**

The optimized eudragit coated sodium alginate microsphere showed a faster onset of anti-inflammatory activity as compare to the control (1% CMC), indicating maximum inhibition of edema. A 78% inflammation inhibition in the eudragit coated sodium alginate microsphere was obtained after 18 h, whereas for pure ACF and marketed ACF, a 41% and 48% inflammation inhibition were observed after 18 h respectively. A highly significant (p<0.05) anti-inflammatory action of the treatment of
optimized eudragit coated sodium alginate microsphere was evidenced by inhibition of rat paw edema as compared to pure ACF and marketed ACF (Table 4).

CONCLUSION
The eudragit coated Sodium alginate microspheres of aceclofenac showed extended release of drug that would keep the blood levels of drugs remain constant throughout the delivery period, which is particularly important for ideal delivery of drug in pain management. The prepared coated microspheres exhibited maximum release of drug in intestinal region at pH 7.4 by zero order kinetics. A highly significant antiinflammatory action of optimized coated microsphere was evidenced by inhibition of rat paw edema.

Conflict of Interest Statement
We declare that we have no conflict of interest.

ACKNOWLEDGEMENTS
This project was supported by Hamdard National Foundation, Hamdard University, New Delhi, India.

REFERENCES


[18] Winter CA, Risley EA and Nuss GW, Carrageenin-induced edema in hind paw of the rat as an assay for


Table 1: Composition of Optimized Core Formulation of Sodium Alginate

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Formulae</th>
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<tr>
<td></td>
<td>F1</td>
</tr>
<tr>
<td>Sodium alginate</td>
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<tr>
<td>Liquid paraffin</td>
<td>40 ml</td>
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<tr>
<td>Drug</td>
<td>250 mg</td>
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<tr>
<td>Drug : polymer ratio</td>
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<tr>
<td>Calcium chloride</td>
<td>7.5 %</td>
</tr>
<tr>
<td>Cross linking time</td>
<td>5 min</td>
</tr>
<tr>
<td>% Yields</td>
<td>87.1 %</td>
</tr>
<tr>
<td>Entrapment efficiency</td>
<td>85.2 %</td>
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Table 2: Composition of coated Microsphere of Sodium Alginate

<table>
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<tr>
<th>Form code</th>
<th>Core (F2)</th>
<th>Coat composition</th>
<th>Parameters</th>
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<tr>
<td></td>
<td>RS:RL</td>
<td>Core: Polymer</td>
<td>Dispersing medium (ml)</td>
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<td>F2C1</td>
<td>100 mg</td>
<td>2.3:1</td>
<td>1:2</td>
</tr>
<tr>
<td>F2C2</td>
<td>300 mg</td>
<td>2.3:1</td>
<td>1:2</td>
</tr>
<tr>
<td>F2C3</td>
<td>500 mg</td>
<td>2.3:1</td>
<td>1:2</td>
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Table 3: Evaluation of Uncoated and Coated Alginate Microsphere

<table>
<thead>
<tr>
<th>Code</th>
<th>% Yield</th>
<th>Particle size (µm)</th>
<th>Polydispersity Index</th>
<th>% EE</th>
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<tbody>
<tr>
<td>F1</td>
<td>87.2</td>
<td>88.6 ± 8.5</td>
<td>0.015</td>
<td>85.3</td>
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<tr>
<td>F2</td>
<td>90.1</td>
<td>88.5 ± 9.0</td>
<td>0.010</td>
<td>90.2</td>
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<tr>
<td>F3</td>
<td>89.4</td>
<td>88.9 ± 9.3</td>
<td>0.021</td>
<td>80.1</td>
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<td>F2C</td>
<td>88.4</td>
<td>686.0 ± 9.3</td>
<td>0.900</td>
<td>90.1</td>
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Table 4: Inhibition of Rat Paw Edema by Sustained Release Microspheres Formulation of ACF Pure Powder and Conventional Tablet Dosage

<table>
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<tr>
<th>Treatments</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>6</th>
<th>12</th>
<th>18</th>
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<tr>
<td>ACF Pure</td>
<td>16.2</td>
<td>22.0</td>
<td>35.3</td>
<td>36.2</td>
<td>39.6</td>
<td>41.2</td>
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<td>ACF Marketed</td>
<td>24.7</td>
<td>28.1</td>
<td>34.6</td>
<td>42.4</td>
<td>43.2</td>
<td>48.5</td>
</tr>
<tr>
<td>F2C</td>
<td>30.2</td>
<td>35.0</td>
<td>55.3</td>
<td>65.2</td>
<td>68.1</td>
<td>78.2</td>
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</table>
Figure 1: SEM Photograph of Microspheres

Figure 2: DSC Thermogram of Aceclofenac and Aceclofenac Microsphere

Figure 3: In-vitro Drug Release Study of Aceclofenac From Optimized Formulation F2C at pH1.2 with 30% PEG, pH 6.8 and pH 7.4 Using Gradual pH Changing Method
Figure 4: Release Kinetics for Optimized Eudragit RS100 Coated Sodium Alginate Microspheres of Aceclofenac